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Effect of Seed Treatment on Physiological Traits of Two Safflower Cultivars under Defoliation

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ABSTRACT

Introduction: To evaluate the effects of priming and defoliation on some physiological traits of two safflower cultivars, a factorial experiment was conducted as a randomized complete block design with three replications in the research farm of Agricultural Sciences and Natural Resources University of Khuzestan in the crop years of 2017 and 2018.
Materials and Methods: The experimental factors consisted of two cultivars of safflower (Esfahan local and Sofeh cultivars) and three levels of priming with [Salicylic acid (SA) 0.9 milli molar, polyethylene glycol (PEG)- 6000 (-10 MPa)] and control at two defoliation levels (without defoliation and 50% defoliation in lower parts of plant).
Results and Discussion: The results showed that the defoliation and priming treatments had significant effects on chlorophyll a, chlorophyll b, and carotenoid content, in such a way that the priming with salicylic acid and polyethylene glycol was associated with a significant decrease in chlorophyll a (34.54%) and chlorophyll b (42.59%); although, the content of carotenoid increased in defoliation and priming with PEG (38.22), carotenoid was decreased in the treatment of defoliation and priming with salicylic acid. Defoliation treatment significantly (28.11%) increased the activity of ascorbate peroxidase in comparison to the control.
Conclusion: Generally, the results showed that the highest enzyme activity of ascorbate peroxidase (29.26%) was observed in the first year of priming with polyethylene glycol. The highest amount of catalase activity (27.45%) was also observed in Isfahan local cultivar and salicylic acid priming under 50% defoliation. The amount of Malondialdehyde decreased in priming with PEG, however, it increased (33.1%) in priming with salicylic acid and 50% defoliation treatment.

Introduction

Safflower (*Carthamus tinctorius* L.) is a member of the family *Compositae* cultivated for its seed, which is used as edible oil. This crop was also grown for its flowers, used for coloring and flavoring foods and making dyes. It is a rich source of oil (35-40 %) and linoleic acid content (75-86 %). Safflower flowers are known to have many medicinal characteristics for curing several chronic diseases. This annual *Compositae* plant is native in Iran, India, the Middle East, and East Africa, and it has recently received much attention due to its adaptation to arid and dry areas in terms of oil production (Poordad, 2003). It has also been proved that the growth and performance of crops in many parts of the world are affected by biotic and non-biological stresses; therefore, there is a large difference between the real and the potential yields. Considering Iran's climatic features, the selection of plants with low water demand and high value added is essential to ensure food security and promote development.

This plant is a multifunctional crop due to its wide adaptation to different climates and its high tolerance to adverse environmental conditions; therefore, conducting extensive breeding and agronomic studies and striving to develop cultivation and improvement are essential (Khajehpour, 2012). In plants, leaves are the main source of light absorption and conversion of the light energy to photosynthetic material for growth, development and filling of seeds, so any reduction in the leaf surface area like diseases, weeds or mechanical damage (hail) can cause malfunction and decreased absorption and utilization of Co_2 in plant (Ashly et al., 2002). Improvement in crop yield was the result of greater dry matter allocation to grains, so approaches to improve grain yield include increased seeding, delayed leaf aging, and increased seed filling duration (Emam and Seghatoleslami, 2005). However, in the first place, crop yield improvement requires seed germination and proper seedling establishment because it exposes plants to a variety of biological and non-biological stresses, especially drought stress. Priming is one of the cheapest ways to improve seedling establishment (Ashraf and Foolad, 2005). Effects of priming on seed stability for better germination have been thoroughly described under suboptimal conditions studied by (Vahid et al., 2007), and the effect of temperature and humidity was investigated by (Dual and Tuong, 2002) in which different methods of priming were completely discussed. It has been reported that the pretreated seeds had high resistance to stress and that seed priming improves the quality and quantity of the crop produced under appropriate and stressful conditions (Read and Kar, 1994). Environmental stresses such as drought, can reduce growth and photosynthetic ability of the plant. Plants usually respond to stress at molecular, cellular and physiological levels. Plants' response to stress depends on the species and genotypes (Rampino et al., 2006), duration and severity of stress (Battaglia et al., 2008), developmental stage and age of the plant (Zhu et al., 2005), body and cell type and subcellular structure (Battaglia et al., 2008). One of the main causes of the damage under stress conditions is the production of all kinds of free oxygen radicals. The presence of reactive oxygen radicals can destroy DNA, RNA, and vital enzymes that are called oxidative damage (Ashraf and Ali, 2008). Malondialdehyde is the product of peroxidation of unsaturated fatty acids in plant cells; therefore, it is used as a suitable biomarker for the determination of the amount of lipid peroxidation induced by oxidative stress in the cell (Sofa et al., 2004). However, the use of some compounds, including growth regulators, increases crop growth and yield by reducing the effects of environmental stresses (Hajihashemi et al., 2007; Dat et al., 1998). Salicylic acid with the effect on antioxidant enzymes such as catalase, superoxide dismutase, polyphenol oxidase and peroxidases, and metabolites such as ascorbic acid and glutathione can reduce the effects of drought, heat, cold, salinity, heavy metals and plant diseases (Coronado et al., 1998). Chlorophyll a and b molecules, along with carotenoids, are the most important light-absorbing pigments in plants (Scheer, 2004). Carotenoids are capable of capturing high energy of short wavelengths and convert single oxygen to triple oxygen. They can also play an antioxidant role by taking the produced oxygen radicals (Amanulla, 2010). Safflower *(Carthamus tinctorius. L)* along with other seed crops is planted in dry countries like Iran since long ago for its edible oil, especially in Khuzestan province south west Iran. This study attempts to investigate the germination behavior, growth and yield of safflower under the effect of priming and defoliation.

Materials and Methods

This research was carried out on safflower plant in Khuzestan University of Agricultural Sciences and Natural Resources Research Field (35 km northeast of Ahvaz, 52°48' N36°31'E and 20m above sea level) in 2016-2017 and 2017-2018 growing seasons. The physical and chemical properties of the soil are shown in table 1 and the meteorological data for two growing years are presented in table 2.

The experiment was conducted as a factorial experiment in a completely randomized block design with three replications. Experimental factors included two safflower cultivars (Sofeh and Isfahan locality), three priming levels (control, 0.9 mM salicylic acid (Badpa et al., 2016), and polyethylene glycol 6000 with -10 MPa osmotic potential (Shatpathy et al., 2018) and defoliation at two levels (Control without defoliation, 50% defoliation in lower part of plant), at 50% flowering stage, manually repeated over two years. For priming, the seeds were immersed in salicylic acid and polyethylene glycol solutions for 25 h at 12 ° C. The plots were manually prepared and each plot consisted of five rows with dimensions of 3*2.5m, spacing of 50 cm, plant spacing of 10 cm and planting depth of 4 cm. There was a one-meter gap between the two replicates. Each replicate had 18 plots and a total of 54 plots in each replicate was present. Fertilizing treatments were determined based on the need of the plant and the amount of soil nutrients. Chemical fertilizers including pure nitrogen 70 kg ha-1, pure phosphorus 50 kg /ha and pure potassium 50 kg/ ha were used. Operations consisted of thinning (in two four- and six-leaf stages), weeding and top dress manure. Measurements were made at the flowering stage (two weeks after defoliation).

Photosynthetic pigments assay

The Lichtenthaler (1987) method was used to measure chlorophyll and carotenoids. Accordingly, 0.2 g of fresh plant leaves were grinded in a Chinese mortar containing 15 ml of 80% acetone and, after passing through the filter paper, the optical density of the extracts were estimated with a Spectrophotometer Model (Unico-2100 Uv / visible) in 663, 645, and 470 nm wavelengths. The amounts of chlorophyll a, chlorophyll b and carotenoids were calculated using the relations 1, 2 and 3.

Eq. 1) Chlorophyll a= $(12.25\ A_{663} - 2.79\ A_{645})$

Eq. 2) Chlorophyll b= $(21.5\ A_{645} - 5.1\ A_{663})$

Eq. 3) Carotenides= $(1000\ A_{470} - 1.82\ \text{chl a} - 82.25\ \text{chl b})/1$

In the above relations, A is the absorption intensity at the corresponding wavelengths in nanometers.

Preparation of Enzymatic Extract

To prepare the enzymatic extract, 0.05 g of fresh leaves was crushed in a Chinese pestle mortar containing 5ml of 50 milli molar Tric-Hcl buffer, pH 7.5. After 20 min of resting, the resulting mixture was centrifuged at 10000 rpm at 4°C for 20 minutes. The supernatant solution was then extracted as an enzymatic extract and stored in a freezer at -20°C.

Ascorbate peroxidase assay

Ascorbate peroxidase activity was measured by Nakano and Asada (1987) by its ability to oxidise H2O2 in plants. Ascorbate peroxidase enzyme activity was determined based on oxidation of H2O2 at 290 nm wavelength, and using a 2.8 mM-1cm-1 extinction coefficient.

Table 1. Physical and chemical properties of soil at a depth of 0-30 cm

Soil texture	Electrical conductivity (dS/m)	pH	Absorbable nitrogen (%)	Absorbable phosphorus (ppm)	Absorbable potassium (ppm)	Organic carbon (%)	Clay (%)	Silt (%)	Sand (%)
Silt clay	3.6	7.4	0.05	7.2	214	0.76	44	40	16

Table 2. Meteorological Statistics of Ahvaz Station during Experimental Period (2016-17 and 2017-18)

Month	January		February		March		April		May		June	
Year	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Precipitation (mm)	2.2	32.6	17.9	33.9	14.6	78.2	0.8	24.1	0.0	26.7	0.0	0.0
Average temperature (ºC)	14.3	14.9	13.2	15	16.1	19.5	23.2	23.6	30.9	27.7	34.2	34.7

Catalase enzyme activity assay

Catalase activity was measured spectrophotometrically using Chance method and Maehly (1995) by measuring the absorption reduction at 240 nm. For this purpose, 2.95 ml reaction buffer including 50 mM potassium phosphate buffer (pH = 7) and 15 mM hydrogen peroxide were mixed with 50 µL of the enzyme extract. The specific activity of the catalase was calculated by the volume of the catalase activity divided by the amount of the protein determined in the enzyme extract.

Malondialdehyde assay

Malondialdehyde concentration was based on the reaction of the enzyme with thiobarbituric acid, producing a red complex, estimated at 532 nm, with a spectrophotometer. Then, the absorbance of the other nonspecific pigments was measured at 600 nm, and the absorbance was subtracted at 532 nm. The Malondialdehyde amount was calculated based on the extinction coefficient 155 mM-1cm-1, and finally the amount of Malondialdehyde was achieved by micromole per gram of the fresh weight.

Statistical calculations

Data were analyzed using SAS software (Ver 9.3), and the mean comparisons were made by LSD test at 5% probability level.

Results and Discussion

Photosynthetic pigment content: The results of the analysis of the variance showed that the simple effect of the defoliation and priming treatments on the chlorophyll a content was significant. In addition to the simple effects of the defoliation and priming treatments, the interactive effect of defoliation and priming was significant on the chlorophyll b content. Regarding the leaf carotenoid content, the simple effects of priming and defoliation were also significant (Table 3). Analysis of the variance results showed that the effect of the defoliation and priming treatments on the chlorophyll a content was significant (Table 3).

For chlorophyll b, the results of the comparison of mean interaction of defoliation * priming treatments showed that the highest content was seen in control priming in the absence of defoliation conditions (Table 4). The lowest chlorophyll b was obtained in 50% of the defoliation treatment with the salicylic acid treatment (Table 5). In addition to the simple effects of the defoliation and priming treatments, the interaction of defoliation * priming on the chlorophyll content was significant (Table 6).

Table 3. Analyses of variance of measured traits under different levels of priming and defoliation in two safflower cultivars

Source of changes	Freedom degree	Malondialdehyde	Ascorbate Peroxidase	Catalase	Carotenoid	Chlorophyll b	Chlorophyll a
Year	1	0.304862^{ns}	0.000390^{ns}	0.001042^{ns}	0.0130414^{s}	0.001012^{ns}	0.0001288^{ns}
Year × Replication	4	0.036512^{ns}	0.000327^{**}	0.013522^{ns}	0.0244366^{ns}	0.005104^{ns}	0.0632486^{ns}
Cultivar	1	0.039573^{ns}	0.000082^{ns}	0.029474^{ns}	0.0013475^{ns}	0.009568^{ns}	0.0156055^{ns}
Year × Cultivar	1	0.002118^{ns}	0.001082^{ns}	0.000726^{ns}	0.0057095^{ns}	0.001012^{ns}	0.0000055^{ns}
Priming	2	0.0405016^{**}	0.016123^{**}	0.007288^{ns}	0.1365846^{**}	0.1155875^{**}	0.8114541^{**}
Cultivar × Priming	2	0.051590^{ns}	0.002197^{ns}	0.003580^{ns}	0.0001560^{ns}	0.0019013^{ns}	0.0073097^{ns}
Year × Priming	2	0.1354659^{ns}	0.000730^{*}	0.006803^{ns}	0.0091731^{ns}	0.0030875^{ns}	0.0326347^{ns}
Year × Cultivar × Priming	2	0.0402304^{ns}	0.001952^{ns}	0.000544^{ns}	0.0021349	0.0077791^{ns}	0.013513^{ns}
Defoliation	2	0.4915605^{**}	0.031649^{**}	0.009401^{ns}	0.1269431^{**}	0.2233472^{**}	1.4734722^{**}
Cultivar × Defoliation	2	0.0104211^{ns}	0.000060^{ns}	0.000004^{ns}	0.0000028^{ns}	0.0021125^{ns}	0.0000500^{ns}
Priming × Defoliation	4	0.0099430^{ns}	0.000216^{ns}	0.002019^{ns}	0.0100079^{ns}	0.0730097^{**}	0.1760013^{s}
Cultivar × Priming × Defoliation	4	0.0008650^{ns}	0.000600^{ns}	0.019559^{*}	0.0010012^{ns}	0.0103125^{s}	0.0006125^{ns}
Year × Defoliation	2	0.0123719^{*}	0.002135^{ns}	0.001201^{ns}	0.00031524^{ns}	0.0141680^{ns}	0.0000500^{ns}
Year × Cultivar × Defoliation	2	0.0010458^{ns}	0.0001344^{ns}	0.003887^{ns}	0.0001221^{s}	0.0001125^{ns}	0.0000055^{ns}
Year × Priming × Defoliation	4	0.0085952^{ns}	0.000533^{ns}	0.004932^{ns}	0.1111195^{ns}	0.0037763^{ns}	0.0059041^{ns}
Year × Cultivar × Priming × Defoliation	4	0.0213465^{ns}	0.004013^{ns}	0.004563^{ns}	0.0001816^{s}	0.0060125^{ns}	0.0108097^{ns}
Error		3.2094513	0.1369017	0.348010	0.8260334	0.006012	5.2547500
Coefficient of Variation		13.5714	14.60680	21.37639	13.54207	14.50199	25.9796

ns, * and ** indicate no significance and significance at 5 and 1% probability levels, respectively.

Table 4. Mean comparison of some of traits under defoliation treatment

Defoliation%	Ascorbate peroxidase mol ascorbate oxidized min^{-1} mg^{-1} protein	Carotenoids mg/g FW	Chlorophyll a mg/g FW
0	0.0414^{b}	0.259^{b}	0.828^{a}
50	0.053^{a}	0.358^{a}	0.542^{b}

The presence of at least one common letter in each column means that there is no significant.

Table 5. Mean comparison of some traits affected by priming treatment

Priming	Malondialdehyde (μmol/g)	Carotenoids (mg/g FW)	Chlorophyll a (mg/g FW)
Control	0.913^{c}	0.306^{b}	0.857^{a}
Salicylic acid	1.530^{a}	0.228^{c}	0.492^{c}
polyethylene glycol	1.216^{b}	0.392^{a}	0.707^{b}

The presence of at least one common letter in each column means that there is no significant difference between treatments.

Table 6. Comparison of the mean of the priming * defoliation interaction on chlorophyll b

Priming	0.0 defoliation	mg/g FW
Control	0	0.620^{a}
	50	0.315^{c}
Salicylic acid	0	0.293^{c}
	50	0.276^{c}
Polyethylene glycol	0	0.429^{b}
	50	0.324^{c}

The presence of at least one common letter in each column means that there is no significant difference between treatments.

According to the results of mean comparison, 50% defoliation treatment increased 38.22% carotenoid content compared to the control treatment (Table 4). In addition, according to the results of the mean comparison data, the highest carotenoid content was related to the polyethylene glycol priming treatment and the lowest amount was related to the salicylic acid priming treatment (Table 5).

The experiment showed that defoliation, PEG and SA priming treatments decreased chlorophyll content (Table 6). The reduction in chlorophyll content under priming conditions might be due to the oxidative stress in such conditions. Changes in lipid compounds and thylakoid membrane proteins might be due to thylakoid and chlorophyll degradation in thylakoid membrane resulting from the plant exposure to drought stress (Syros et al., 2005). In chickpea genotype experiments, it was proved that under drought stress, the amount of chlorophyll a and b decreased (Sabokdast Nodehi and Khialparast, 2007). Decreases in chlorophyll content have been reported in studies (Ahmadi and Seose Marde, 2004) and (Terzi et al., 2010). However, it has been reported that salicylic acid has a positive effect on the plant metabolism by increasing their chlorophyll content or biosynthesis in plants exposed to drought stress as well as by affecting plant metabolic reactions. These changes are often in the form of adaptations that increase the plant drought tolerance. It has also been reported that photosynthetic pigments in maize were increased with the application of salicylic acid (Azooz et al., 2011). However, salicylic acid has dual effects depending on the concentration, time and plant used, which can either degrade chloroplast proteins and lipid peroxidation of thylakoid membranes by increasing active oxygen species and reduction of photosynthetic pigments. At appropriate concentrations, SA could support the photosynthesis system which reduces the degradation of chlorophyll pigment (Belkhadi et al., 2010), which leads to an increase in cell antioxidant resistance capacity and synthesis of new proteins (Popova et al., 1997). It has been reported that the increase in carotenoid content could improve the quality of photosynthesis, as a result, it helps to enhance the antioxidant resistance capacity. This pigment is responsible for extinguishing single oxygen production and preventing lipid peroxidation during oxidative stress (Koryo, 2006). The results of this experiment were consistent with the findings of (Lee et al., 2005; Abdalla and El-Khoshiban, 2007). The researchers also reported that under the environmental stress, the content of carotenoid increased significantly and played a protective role for chlorophylls and prevented the degradation of chlorophylls under severe drought and light stress.

Antioxidant enzymes

Ascorbate Peroxidase

Analysis of the variance results indicated that, the simple effects of priming and defoliation treatments, as well as the interaction of priming and year treatments were significant (Table 3). The results of mean comparison showed that the defoliation treatment increased ascorbate peroxidase activity significantly (28.11%) compared to the control (absence of defoliation). Moreover, according to the results of the comparison of the mean interaction between priming treatment and year, the highest amount of the ascorbate peroxidase activity was related to the first year treatment with polyethylene glycol priming treatment. However, there was no significant difference between salicylic acid and polyethylene glycol priming treatment in the second year (Table 7).

Aerobic metabolism in plants results in the generation of reactive oxygen species (ROS). ROS are produced constantly in plants under physiological steady state condition, and plants have evolved to efficiently scavenge and maintain the levels of ROS at non-damaging levels. However, plants, when exposed to either abiotic or biotic stress conditions, the production of ROS exceeds their scavenging capacity, leading to an outburst of highly reactive oxidative species capable of inflicting significant damages to the membranes, DNA, and proteins. On the other hand, these reactive molecules, when maintained under non-damaging levels, are useful signaling molecules involved in the relaying stress signal to activate acclimation and defense mechanism. Drought or water deficit stress is one of the major abiotic stresses which induces the production of different kinds of ROS (Valko et al., 2005). Plants have antioxidant defense mechanisms to counteract these reactive oxygen species. In this study, it was found that defoliation and priming with polyethylene glycol and salicylic acid significantly increased the activity of the antioxidant enzymes compared to the control; it might be as a result of oxidative stress in such conditions to reduce stress induced damages. Physiological and genetic evidence suggests that the plant's antioxidant system is an important component of stress protection mechanisms (Sairam et al., 1998). Antioxidant enzymes and metabolites create a high-performance system that inactivates ROS and reduces their damage. Ohe et al., (2005) showed that during drought stress, the activity of oxidizing superoxide dismutase enzymes, ascorbic peroxidase and catalase increased in spinach and tobacco leaves. However, the decreased activity of the antioxidant enzymes has also been reported in some studies. For example, a decrease in the activity of some antioxidant enzymes has been reported in some wheat species. These differences appear to be due to the type of plant species, the stage of development of plant tissue and environmental conditions (Kasano et al., 1994). Concerning the role of salicylic acid in stress conditions, several studies have recently demonstrated the practical role of salicylic acid as an important messenger molecule in the fluctuations of plant responses to multiple stresses (Khan et al., 2003).

Table 7. Mean comparison of ascorbate peroxidase affected by priming treatment in two years of experiment

Year	Priming	Ascorbate peroxidase Micromole of H2O2 decomposed per minute in mg protein
First	Control	0.035[b]
	Salicylic acid	0.042[b]
	Polyethylene glycol	0.062[a]
Second	Control	0.034[b]
	Salicylic acid	0.053[a]
	Polyethylene glycol	0.56[a]

The presence of at least one common letter in each column means that there is no significant difference between treatments.

The results of this experiment showed that the activity of antioxidant enzymes was significantly affected by the salicylic acid application, so that salicylic acid increased the activity of these enzymes significantly. Salicylic acid has also been reported to affect antioxidant enzymes such as catalase (Slaymarker et al., 2002), superoxide dismutase and polyphenol oxidase (Dat et al., 1998), and peroxidases (El-Tayeb, 2005); and metabolites such as ascorbic acid (Slaymarker et al., 2002; Borsani et al., 2001), and glutathione (Borsani et al., 2001) reduce the effects of drought (Senaratna et al., 2003) and heat (Dat et al., 1998). However, the improvement of some physiological processes and the inhibition of some processes depend on the concentration of salicylic acid, plant species, different stages of development, and environmental conditions (Mateo et al., 2003). Higher Salicylic acid concentrations affect the oxidative state of the plant over the power of the plant and ultimately lead to plant death (Kovacik et al., 2009). Not only Salicylic acid at a concentration of 1 mM did not decrease the negative effects of salinity stress on the growth, lipid peroxidation, ion leakage, and photosynthetic pigments, but also increased the stress itself (Danesh et al., 2011). Also, regarding the variations of plant responses to the treatments applied in different years in this study, it seems that in the first year due to climatic conditions such as higher temperature, lower rainfall and increased heat radiation and under priming conditions, polyethylene glycol caused drought stress and, consequently, increased the activity of the antioxidant enzymes. In contrast, in the second year, due to more climatic and rainfall conditions, in the control (non-priming) conditions the plant was not stressed and the heat radiation was lower due to the cloudier days; therefore, the antioxidant enzymes activity decreased.

Malondialdehyde

According to the results of the analysis of variance, the simple effects of priming and defoliation, and the interaction effects of year and defoliation on the content of malondialdehyde were found to be significant (Table 3). Results of the mean comparison showed that the highest content of malondialdehyde was related to salicylic acid priming and the lowest was in the control priming condition. In addition, according to the results of comparing the mean interaction effect of year and defoliation, the highest content of malondialdehyde was related to the first year under 50% defoliation treatment. In contrast, although the lowest content of malondialdehyde was in the first year and without defoliation treatments, it was not significantly different with 50% and the absence of defoliation treatments in the second year (Table 8).

Plant cells are surrounded by cell membranes. One of the unique features of the membranes is their selective permeability, which prevents the cell-to-cell equilibrium and the outer environment. However, drought stress alters the plant cell equilibrium, which may be due to the increased production of reactive oxygen species (ROS). Increased reactive oxygen content induces lipid peroxidation (LPO) of the cellular membrane (Beckers and Spoel, 2006). Based on the results obtained in this experiment, it was found that malondialdehyde content increased under the salicylic acid priming as well as 50% treatment. In this experimental condition, the salicylic acid priming and defoliation treatment causing oxidative stress, were followed by other biochemical reactions related to it, including increased malondialdehyde content. It has been reported that oxygen free radicals with peroxidation of unsaturated fatty acids produce aldehydes such as malondialdehyde (MDA). The Malondialdehyde content is the second by-product of unsaturated fatty acid oxidation and generally measures lipid peroxidation as an index of the oxidative stress to assess the extent of membrane damage under stress conditions (Watanabe et al., 2000).

Table 8. Mean comparison of malondialdehyde affected by the interaction of defoliation and year

Year	Defoliation (%)	Malodealdehyd (μmol/g)
First	0	1.00[b]
	50	1.52[a]
Second	0	1.097[b]
	50	1.24[b]

The presence of at least one common letter in each column means that there is no significant difference between treatments.

Although salicylic acid priming increased the content of malondialdehyde in this experiment, some reports suggest that using salicylic acid in combination with drought may increase some physiological processes that can increase the plant resistance to drought stress (Arvin, 2011). Moreover, decreased lipid peroxidation of membrane has been reported by salicylic acid under drought stress conditions (Keshavarz et al., 2011). However, and in line with the results of the present study, it has been reported that high concentrations of salicylic acid damage biological membranes resulting from the production of radicals over the plant defense capacity due to the effect of salicylic acid (Mahdavi et al., 2011). Application of salicylic acid resulted in accumulation of H2O2 in rice plant (Jiang and Huang, 2002). The researchers concluded that the use of salicylic acid could lead to oxidative stress.

Conclusion

This experiment showed that defoliation, salicylic acid priming and polyethylene glycol decreased the chlorophyll content and increased the antioxidant enzymes activity compared to the control treatment. In contrast, the defoliation treatment and priming of polyethylene glycol increased the carotenoid content. The results also showed that the malondialdehyde content was increased under the salicylic acid priming as well as 50% defoliation treatment. Therefore, according to the results obtained in this experiment, it seems that the use of higher concentrations was not only ineffective in reducing the negative impacts of stress, but may be harmful through oxidative stress. Therefore, according to the results of this study, applying different concentrations of seed priming treatments is suggested for better seedling establishment and further reducing the negative effects of the environmental stresses on yield improvement.

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